ELECTRO- AND MAGNETOBIOLOGY, 13(1), 67-75 (1994)

MAGNETIC PULSES ELEVATE NOCICEPTIVE THRESHOLDS: COMPARISONS WITH OPIATE RECEPTOR COMPOUNDS IN NORMAL AND SEIZURE-INDUCED BRAIN-DAMAGED RATS

Jennifer L. Fleming, M. A. Persinger, and Stanley A. Koren
Behavioral Neuroscience Laboratory
Department of Psychology
Laurentian University
Sudbury, Ontario, Canada P3E 2C6

ABSTRACT

Normal rats or rats with seizure-induced limbic-thalamic damage were given one of five treatments: control, saline injections, morphine (4 mg/kg) injections, naloxone (10 mg/kg) injections, or magnetic pulses, on 3 successive days. Flinch thresholds to electric shock were determined before the treatments and 20 and 40 min following the treatments on each of the 3 days. The results indicated that the magnetic pulsed fields (1 s of a 5×10^{-6} T burst firing pattern every 4 s for 20 min) elicited a more prolonged and larger analgesic effect than the other treatments; the effect size was sufficient to be of potential clinical relevance. The characteristics of the magnetic treatment effect suggest there is a release of endogenous analgesics whose half-life is greater than experimental dosages of morphine.

Send reprint requests to M. A. Persinger, Behavioral Neuroscience Laboratory, Department of Psychology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6.

Copyright © 1994 by Marcel Dekker, Inc.

INTRODUCTION

Magnetotherapies have been employed to treat both the experience of and the response to nociceptive (painful) stimuli (1). The neuromechanisms by which changes occur are not clear because of: (a) the multiple pathways, and hence types of nociceptive experiences and responses (2), and (b) the arbitrary and often unspecified characteristics of the field parameters (1). Although most magnetic field devices allow changes in the frequency (usually 1 Hz to 100 Hz), they primarily employ redundant sinusoidal signals that are presented continuously during treatment.

The release of endogenous analgesics, such as the enkephalins or the endorphins, can be encouraged by specific temporal patterns in sensory stimulation. Lewis *et al.* (3) reported that, whereas a single episode of foot shock did not alter nociceptive thresholds, the presentation of shock for 1 s every 4 s significantly elevated these thresholds. The effect was partially blocked by naloxone, a well-known mu-receptor antagonist, and suggested involvement of these morphine receptors.

Ultimately all somatic sensory input reaches the ventrobasal complex of the thalamus where there is correlative activation of the parafascicular-centromedian region and the mediodorsal complex (4). The somatosensory, arousal, and affective dimensions of nociception emerge from the differential contributions of each of these thalamic zones (5). Alteration in the ratio of thalamic neuronal activity between sensory (afferent) volleys and the interneuronal (baseline) activity determines the intensity of the pain response.

Pain responses and experiences can be amplified by: (1) increasing the magnitude of the sensory volleys (a normal physiological sequence) or (2) decreasing the activity of the interneurons. Loss of these thalamic neurons is a common sequela to the transneuronal degeneration that follows many cases of traumatic brain injury or closed head injury (6). The loss of inhibitory interneurons would encourage even normal sensory information to be experienced as pain and could constitute the neuroelectrical correlate to the thalamic pain syndrome.

Because all sensory input is ultimately translated into temporal patterns of action potentials, one could assume that induction of these electrical patterns by the application of magnetic fields could generate similar changes in pain thresholds. We hypothesized that the whole-body application of the appropriate magnetic field [whose temporal pattern was very similar to that of the foot shock sequence required to invoke analgesia (3)] should also elevate the pain threshold. To discern if the temporal characteristics and strength of the effect were similar to those of the well-known analgesic morphine, this drug and its direct antagonist naloxone were employed as comparators.

The potency of some analgesics is maximally effective following the first application but then attenuates following subsequent treatment (7). To determine the consistency of any analgesic effect from applied magnetic fields, which would be important for treatment of chronic pain disorders, we decided to test nociceptive thresholds over 3 successive days. Finally, because many types of pain are secondary to neuronal loss within the thalamus, rats in which seizure induced damage within the thalamus and limbic system had occurred were also selected as subjects (8,9). Micromorphometric measures have indicated that there is about a 25% loss of neurons within the thalamic regions (e.g., parafascicular nucleus) that are most frequently associated with nociception (10).

MATERIALS AND METHODS

A total of 60 Wistar albino female rats, 120–150 days old, served as subjects. Limbic status epilepticus (forelimb clonus) had been induced in 26 of the rats approximately 30–40 days before the current testing began. The seizures (not convulsions) had been induced by a single systemic (subcutaneous) injection of lithium chloride (3 mEq/kg) followed 4 h later by a single subcutaneous injection of 30 mg/kg of pilocarpine. To ensure the survival of these animals, 25 mg/kg of acepromazine was injected within 60 min after the injection of the pilocarpine, i.e., about 30 min after the average seizure onset time. The neuropathological patterns of the subsequent brain damage, which occurs in 100% of the rats that receive this treatment, have been reviewed elsewhere (11,12).

All rats were housed three per cage within standard colony conditions. The L:D 12:12 hr cycle began at 0730. Food and water were available *ad libitum*. Rats in which seizures had been induced were housed together, and normal rats were housed together.

A similar number of normal and seizured rats were assigned to one of five conditions: control, saline, morphine, naloxone, or magnetic pulsed field. During 3 successive days, each rat was removed from the home cage, brought to the experimental room, and placed in a plexiglass chamber that contained a metal grid floor. The intensity of electric shock was slowly increased (from zero) through an A-615-C Master Shocker (Lafayette Instrument Co.) until the rat displayed a clear flinch; this movement was qualitatively distinct. The intensity was then determined and the rat was immediately removed and returned to its home cage. This threshold was determined 20 min later and then after an additional 20-min interval. Again, this procedure was completed on 3 successive days between 11:00 A.M. and 1:00 P.M.

For each of the 3 days the first threshold (or trial) served as the baseline for the animal. After the first trial on each of these 3 days, each rat received one of the five treatments. Each rat always received the same treatment. Control rats were simply returned to their cage, while the saline rats received i.p. injections of 1 cc/kg of isotonic saline. The two drug groups received either 4 mg/kg of morphine sulfate or 10 mg/kg of naloxone; these dosages were known to produce changes in nociceptive thresholds (13).

The rats that received the magnetic field treatment were placed singly in a 17 cm by 30 cm by 13 cm (deep) plastic cage. Two solenoids (each composed of a 25 cm long by 1 cm diameter soft iron nail wrapped with 1050 turns of 20 gauge insulated wire), whose points were separated by 20 cm, generated the field across the cage. An IBM-compatible personal computer was used to generate the burst-firing (amygdaloid) pattern through Hercules complex software (14) that was connected to the solenoids through a differential (d.c. coupled) amplifier. Because there were 250 successive temporal points in the waveform and we selected 4 ms as the duration of each point, the pulse duration was 1 s. The time between pulses was 3000 ms. Consequently the field, which was measured by a Metex N3800 meter coupled to a magnetic sensory, pulsed at $1\text{–}5 \times 10^{-6}$ T (5×10^{-6} T within approximately 2/3 of the volume of the cage) for 1 s every 4 s. Daily exposure time was 20 min.

The experimental design was a four-way analysis of variance with two levels repeated. The two independent levels were the five treatments and the two rat sources (normals vs. rats with seizure-induced neuronal loss). The two within-subject levels

were the 3 days and the three trials (within each day). Multivariate analysis of variance (MANOVA), using SPSSx software on a VAX 4000 computer, was employed to analyze this design. Covariance for body weight was also completed. Subsequent *post hoc* analyses (Tukey's and selected correlated *t*-tests) were completed by employing appropriate software to discern the source of the effect.

Because of the significant contribution from individual differences, a second MANOVA was completed for the relative change in threshold. The flinch intensity values for trial 2 and trial 3 for each day for each rat were subtracted from the value for trial 1 of each day. This net difference was then divided by the value for trial 1 and multiplied by 100. We called this measure the percentage change in nociceptive threshold. There were two of these values per day, for 3 days.

RESULTS

Repeated measures analysis of variance demonstrated statistically significant differences in mean flinch threshold (in milliamps) between the two groups (control: 0.80 (0.01) vs. seizured: 0.92 (0.02) of rats [$F(1,49) = 6.77, p = 0.01$]) and among the five treatments [$F(4,49) = 6.59, p < 0.001$; eta = 0.56]. *Post hoc* analysis indicated that the major source of the main treatment difference (average of the repeated trials and days) was between the magnetic field-treated group and each of the other

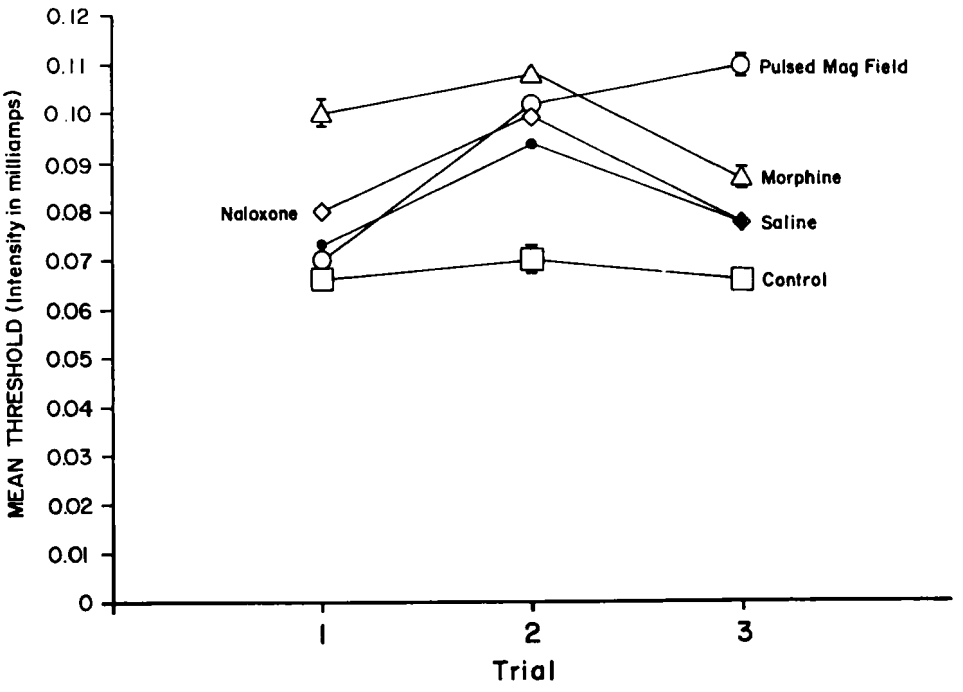

FIGURE 1. Mean flinch threshold (milliamps) over three successive daily trials (each separated by 20 min) for five treatments. Sample standard errors of the means are indicated; group standard deviations ranged between 0.01 and 0.02.

four treatments that did differ significantly from one another. Covariance for body weight before the analysis did not alter the effect size or the level of statistical significance.

Although the repeated measure effect between trials (mean of the 3 days) was statistically significant [$F(2,98) = 15.79, p < 0.001$], the main difference (mean of three trials) between days [$F(2,98) = 0.41, p > 0.05$] was not. The major statistically significant interaction occurred between trial and treatment [$F(8,98) = 5.50, p < 0.001$]. This result is shown in Figure 1. *Post hoc* analyses indicated that the major source of the interaction was due to the maintained elevated flinch threshold (in milliamps) for the magnetic treatment during trial 3 (the last 20-min period) compared to the other treatments (especially morphine), whose efficacy markedly decreased. The second source of the interaction was due to the increase in flinch threshold during the first 20 min that followed the daily treatments for the naloxone, saline,

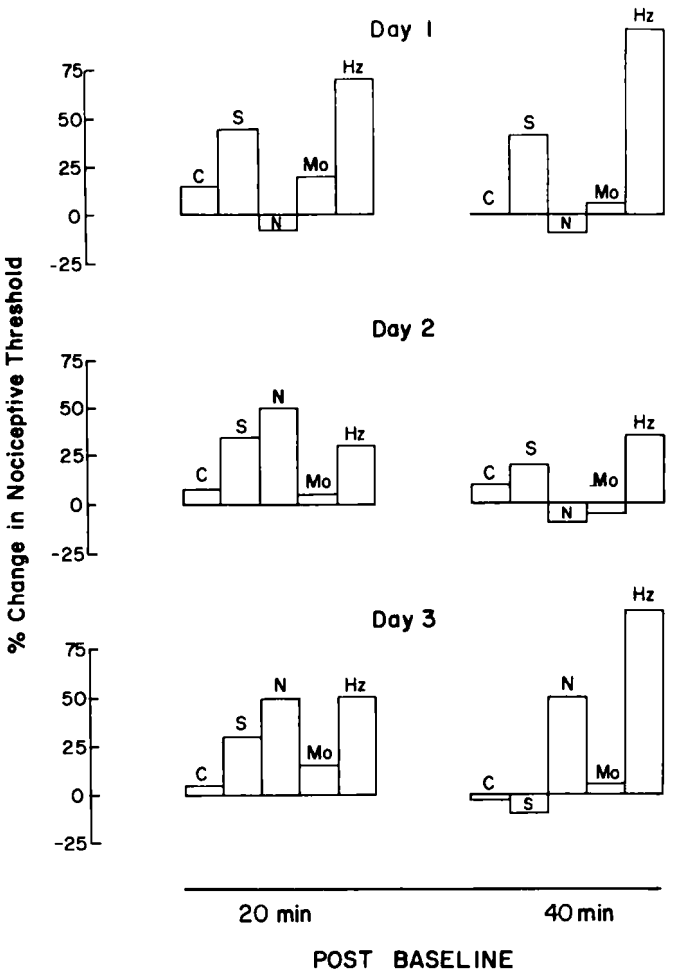

FIGURE 2. Percentage change in nociceptive (flinch) threshold relative to each rat's baseline measure as a function of remaining daily trials, days, and treatment; group standard errors of the means ranged between 8% and 22%.

and magnetic field groups relative to the control treatment. All other interactions were not statistically significant.

The means for the *percent change in nociceptive* threshold (calculated by subtracting thresholds for trial 2 and trial 3 from trial 1, i.e., baseline, dividing by trial 1, and then multiplying by 100) for each treatment, day, and trial are shown in Figure 2. Repeated measure analysis of variance demonstrated a highly significant treatment difference [$F(4,48) = 7.92$, $p < 0.001$; eta = 0.61]; the difference between normal and seizured rats was no longer significant [$F(1,48) = 2.92$, $p > 0.05$]. There was no significant group-by-treatment interaction [$F(4,48) = 0.08$, $p > 0.05$]. *Post hoc* analyses indicated that the major source of the treatment differences was due to the group that received the magnetic field treatment. They displayed a mean 63% (SD = 33%) increase in threshold that was significantly different from each of the four other treatments, which did not differ significantly from each other.

The significant [$F(8,96) = 3.08$, $p < 0.01$] treatment-by-day interaction was due primarily to the maintained elevation in relative analgesia for the magnetic field treatment on the second and third days compared to the other treatments. Similarly, the statistically significant treatment-by-trial interaction [$F(4,48) = 3.98$, $p < 0.01$] was due to the maintained effect of the magnetic field treatment during the second 20-min period for each day compared to all other treatments, whose analgesic efficacy decreased. All the other combinations of repeated measures and interactions were not statistically significant ($p > 0.05$).

DISCUSSION

The results of this study supported the hypothesis that delivery of weak (5×10^{-6} T) magnetic fields for 1 s every 4 s produces an analgesic effect comparable to more noxious tactile stimulation through somatic sensors (3). Rats that were exposed for only 20 min to the 1 s/4 s pulsed field exhibited an approximately 50% increase in the flinch threshold to 60 Hz electric current. The duration and magnitude of the analgesia were greater than those produced by a single injection of morphine.

The validity of the effect is suggested by the general elevation of nociceptive thresholds during the second trial (for each of the 3 days) by groups that were: (1) handled or (2) injected, relative to the nonhandled controls (Fig. 1). Brief stress-induced analgesia is a common phenomenon whose magnitude is comparable to our observations (7). [The elevation of the absolute threshold for the morphone group in Figure 1 would also be expected since the mean of all 3 days (sessions) would have been used in calculation of the blocks of trials.] A single one-way analysis of variance indicated no statistically significant group differences in baseline nociceptive thresholds for the first day *only*; the conspicuous elevation when the daily sessions were averaged may suggest the contribution of a learning factor (21) since the pharmacological continuance of the initial morphine effect is highly unlikely. The relative hyperalgesic effect induced by naloxone on the first day and the subsequent hypoalgesic effects have also been reported previously.

The duration and magnitude of the analgesia that was exhibited by the rats exposed to the pulsed magnetic field strongly implicate the role of the endorphins rather than the enkephalins. Half-lives of the latter neuropeptides are in the order of 2 min whereas the analgesic correlates of (a single i.p. injection of) the endorphins continue

for between 30 min and 60 min (15). Because most of the beta-endorphin-producing neurons are contained within the hypothalamus, the magnetic field pattern should have stimulated neural structures that encouraged cleavage of this component from propiomelancorticotropin or the release of this precursor. If the beta-endorphins are the molecular substrate by which the magnetic field treatment mediated its anti-nociceptive effect, then it should be blocked by naloxone.

One possible mechanism would be the direct effects of magnetic fields on the extremely electrically labile amygdala-hippocampal complex (14). Endogenous electrical activity of these structures, because of their connections with hypothalamic neurons that generate releasing factors, could evoke ventral hypothalamic-hypophyseal activity. Limbic afterdischarges are associated with elevations in circulating prolactin and adrenocorticotropic hormone (ACTH) (16). The strong correlation between levels of ACTH and beta-endorphin indicate that both are fragments from a common precursor molecule. If this is correct, then the 1 s/4 s stimulation should have stimulated the release of endorphins by the current-induced enhancement of amygdalohypothalamic acitivity.

An interaction between brief (30 min) exposures to 0.5 Hz rotating magnetic fields and exogenous opiates has been reported by Ossenkopp, Kavaliers, and their colleagues (17,18). However, they observed an abolishment of the normal nocturnal analgesia (hot plate flinch) if the rodents were first exposed to the magnetic field. Although the magnetic field may have evoked the release of endogenous opiates that competed with somatic analgesic components of the response to thermal stimuli, there is also the possibility of a magnetic-field-induced alteration in receptor-ligand binding. The option that magnetic field changes are actually transient modifications in receptor-binding capacities must still be verified.

The elevated nociceptive threshold that was displayed by rats in which selective brain damage had been induced by lithium/pilocarpine seizures would have been expected in light of the correlative damage in the centromedian and parafascicular nuclei of the thalamus (10). If one assumes that the capacity for the integration of nociceptive input is a function of the numbers of cell bodies within the intralaminar group, then the 25% increase in flinch threshold and the 25-30% necrosis within this thalamic region would be rational. That these changes were permanent in the seizured rats was demonstrated by the elimination of group (normal vs. seizure) differences when each rat was employed as its own control.

However, the absence of a statistically significant interaction between group and treatment suggests that the seizured rats (despite their mean elevated flinch thresholds) responded similar to controls in each of the treatment conditions. These control-like changes indicate that the thalamic centers of the rats in which seizures had been induced might still be responsive to the magnetic field treatment.

It may be relevant that the central nucleus of the amygdala (11), which is the only component of this complex limbic structure that is not observably disrupted by the consequences of the lithium/pilocarpine seizures, is intimately involved with endogenous opiate systems (19). If the central nucleus has been disinhibited by the normal loss of intra-amygdaloid inputs, then the elevated nociceptive threshold in the rats in which seizures had been induced could be due to the results of maintained opiate elevation that is secondary to a subclinical (limbic) status epilepticus. Elevated nociceptive thresholds are frequently measured in schizophrenic syndromes, some of which may be evoked by subclinical partial complex epilepsy (20).

If generalizable to human settings, the results of this study suggest that brief exposure to *weak magnetic fields with the optimal pattern of interstimulus presentation* may evoke antinociceptive benefits to patients. Because many patients with closed head injuries also experience chronic pain, the positive response to this treatment by the brain-injured rats in this study suggests that comparable benefits might be obtained. Subsequent experiments may wish to differentiate the effects of these treatments on the somatic, affective, and arousal components of nociception.

The treatment of pain by either traditional (pharmacological) or magnetic field intervention requires a serious appreciation for the recent experiments of Wiertelak *et al.* (21). Rats placed within situations in which safety has been learned do not display analgesia in response to morphine. There are two antagonistic systems: one that responds to stress, such as the endogenous opiate systems, and one that is antianalgesic and responds to nonstress (safety). If this principle is generalizable, then a large portion of the variability in responsiveness of pain-prone patients to magnetotherapy could be a function of the degree to which the exposure settings are associated with safety and relaxation.

ACKNOWLEDGMENT

The authors thank Yves Bureau and Oksana Peredery for their technical assistance.

REFERENCES

1. Persinger, M. A.: The modern magnetotherapies, in Modern Biolectricity, A. A. Marino, ed., Marcel Dekker, New York, 589-627, 1988.
2. Henry, J. L.: Circulating opioids: possible physiological roles in central nervous function, Neurosci. Biobehav. Rev. *6*, 229-245, 1982.
3. Lewis, J. W., Cannon, J. T. and Libeskind, J. C.: Opioid and nonopioid mechanisms of stress analgesia, Science *208*, 623-625, 1980.
4. Dostrovsky, J. O. and Guilbaud, G.: Nociceptive responses in medial thalamus of the normal and arthritic rat, Pain *40*, 93-104, 1990.
5. Persinger, M. A.: Neurocognitive bases of central pain and depression following traumatic brain injury: eliminating the myth of functional-organic dichotomy, Percept. Motor Skills (submitted).
6. Povlishock, J. T. and Coburn, T. H.: Morphological changes associated with mild head injury, in Mild Head Injury, H. S. Levin, H. M. Eisenberg and A. L. Bento, eds., Plenum Press, New York, 37-53, 1988.
7. Havlicek, V., West, M., Kato, N. and Friesen, H. G.: Beta-endorphin and central nervous system, in Endorphins and Opiate Antagonists in Psychiatric Research, N. S. Shah and A. G. Donald, eds., Plenum Press, New York, 127-189, 1982.
8. Harrigan, T., Peredery, O. and Persinger, M. A.: Radial maze learning deficits and mediodorsal thalamic damage in context of multifocal seizure-induced brain lesions, Behav. Neurosci. *105*, 482-486, 1991.
9. Peredery, O., Persinger, M. A., Blomme, C. and Parker, G.: Absence of maternal behavior in rats with lithium/pilocarpine seizure-induced brain damage: support of MacLean's triune brain theory, Physiol. Behav. *52*, 665-671, 1992.

10. Bureau, Y. R. J. and Persinger, M. A.: Geomagnetic activity and enhanced mortality in rats with acute (epileptic) limbic lability, Int. J. Biometeorol. *36*, 226–232, 1992.
11. Persinger, M. A., Bureau, Y., Kostakos, M., Peredery, O. and Falter, H.: Behaviors of rats with insidious brain damage induced by seizures following single peripheral injections of lithium and pilocarpine, Physiol. Behav. *53*, 849–866, 1993.
12. Lafreniere, G., Peredery, O. and Persinger, M. A.: Progressive accumulation of large aggregates of calcium-containing polysaccharides and basophilic debris within specific nuclei after lithium/pilocarpine-induced seizures, Brain Res. Bull. *28*, 825–830, 1992.
13. Terman, G. W., Shavit, Y., Lewis, J. W., Cannon, J. T. and Liebeskind, J. C.: Intrinsic mechanisms of pain inhibition: activation by stress, Science *226*, 1270–1277, 1984.
14. Persinger, M. A., Koren, S., Makarec, K., Richards, P. and Youlton, S.: Differential effects of wave form and the subject's possible temporal lobe signs upon experiences during cerebral exposure to weak magnetic fields, J. Bioelectricity *10*, 141–184, 1991.
15. Chau, T. T.: The endorphins and analgesia: a minireview, in Endorphins and Opiate Antagonists in Psychiatric Research, N. S. Shah and A. G. Donald, eds., Plenum Press, New York, 41–60, 1982.
16. Gallagher, B. B., Flanigin, H. F., King, D. W., Smith, J. R., Munro, A. M. and Littleton, W.: ACTH secretion and human temporal lobe epilepsy, in Fundamental Mechanisms of Brain Function, J. Engels, G. Ojemann, H. Luders and P. Williams, eds., New York, Raven Press, 201–208, 1987.
17. Ossenkopp, K-P. and Cain, D. W.: Inhibitory effects of acute exposure to low-intensity 60 Hz magnetic fields on electrically kindled seizures in rats, Brain Res. *442*, 255–260, 1988.
18. Kavaliers, M., Ossenkopp, K-P. and Tysdale, D. M.: Evidence for the involvement of protein kinase C in the modulation of morphine-induced "analgesia" and the inhibitory effects of exposure to 60-Hz magnetic fields in the snail, *Capaea nemoralis*, Brain Res. *554*, 65–71, 1991.
19. Dupont, A., Merand, Y., Rouleau, D., Cusan, L., Lemay, A., Vaudry, S., Jegou, S., Lepine, J. and Barden, N.: Relationship of opiate peptides to neuroendocrine functions, in Endorphins and Opiate Antagonists in Psychiatric Research, N. S. Shah and A. Donald, eds., Plenum Press, New York, 99–126, 1982.
20. Ardila, A. and Gomez, J.: Complex partial status and schizophrenia, Int. J. Neurosci. *39*, 235–244, 1988.
21. Wiertelak, E. P., Maier, S. F. and Watkins, L. R.: Cholecystokinin antianalgesia: safety cues abolish morphine analgesia, Science *256*, 830–833, 1992.